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Low level saliva cotinine determination and its application as a biomarker for environmental tobacco smoke exposure

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1 **The determination of personal exposures to environmental tobacco smoke (ETS) and respirable suspended particles (RSP) for housewives, and fixed site monitoring of their homes, have been undertaken by these authors throughout Europe, South East Asia and Australia. Median 24 h time weighted average (TWA) concentrations for ETS particles and nicotine were found to be significantly higher for housewives living in smoking households compared with those living in nonsmoking households. For Europe, median TWA concentrations of 4.1 and <0.26 $\mu g/m^3$ for ETS particles and 0.63 and <0.08 $\mu g/m^3$ for nicotine were found for housewives living in smoking and nonsmoking households respectively.**

2 **In addition to the measurement of RSP, ETS particles and nicotine, saliva cotinine concentrations were determined using a radioimmunoassay method with a limit of quantitation of 1 ng/ml. Median saliva cotinine concentrations of 1.4 and <1 ng/ml were determined for European housewives living in smoking and nonsmoking households respectively, which reflected the poor limit of quantitation of this methodology. A chromatographic method utilising tandem mass-spectrometric detection was developed and validated for the determination of both cotinine and 3-hydroxycotinine, two of the main metabolites of nicotine, with lower limits of quantitation of 0.05 and 0.10 ng/ml respectively. This method was applied to samples collected from subjects with a known ETS exposure history and median cotinine concentrations of <0.05 ng/ml for self-reported unexposed nonsmokers, 0.65 ng/ml for nonsmokers reporting some ETS exposure and 1.28 ng/ml for nonsmokers living with smokers were found.**

3 **In conclusion, the measurement of RSP and ETS concentrations derived from personal or fixed site monitors for housewives may provide some indication of potential exposures to dependent children. The recent development and application of a highly sensitive assay for the determination of cotinine in saliva has provided evidence to suggest that concentrations determined at sub-nanogram levels may be used as a biomarker for ETS exposure. This improved methodology, coupled with non-invasive sampling for saliva, may be of significance when considering the application of cotinine as a biomarker for ETS exposure in children.**

Keywords: saliva; cotinine; nicotine; biomarker; environmental tobacco smoke; personal monitoring

Introduction

Exposure to environmental tobacco smoke (ETS) is an issue of ever increasing concern, particularly with regard to children, as a number of possible adverse health effects have been reported to be related to ETS exposure. An increased risk of cancer and other infectious and developmental impairments,[1,2] as well as respiratory effects and sudden infant death syndrome (SIDS),[3–5] have been reportedly linked with ETS exposure in young children. ETS exposures may be determined using one or more of four principal methods, as summarised by Wu (1997),[6] comprising questionnaire assessment, 'area' or 'fixed site' air monitoring, 'personal' air monitoring and biomonitoring, each with inherent advantages and disadvantages. The use of a biological marker for assessing ETS exposure should provide an indication of actual absorbed ETS 'dose', whereas intake estimated using air concentrations of ETS 'markers' will depend upon breathing rate, mouth *versus* nose breathing and other respiratory factors.

Nicotine is present as a major component of tobacco smoke, and is absorbed and measurable in both actively and passively exposed individuals.[7,8] However, its relatively short half-life ($t_{1/2} \approx 1-2$ h) precludes its use as an accurate marker for ETS exposure since assessments of low level exposure over protracted time periods are often desired. Cotinine, one of the primary metabolites of nicotine, has a much longer half-life ($t_{1/2} \approx 18-20$ h) and is generally considered to be a more appropriate biomarker for evaluating ETS exposure.[7] At present, cotinine is regarded as the biomarker of choice for monitoring tobacco exposure in both active smokers

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and nonsmokers exposed to ETS.[9] However, in recent times the specificity of cotinine as a biomarker has been questioned since dietary sources of nicotine have been identified (e.g. tomato, potato, cauliflower, tea),[10,11] although the contribution of dietary nicotine to serum cotinine levels is estimated to be small in comparison to ETS exposure.[12]

Following exposure to nicotine, cotinine can be found in most body fluids and methods for its determination in blood (serum/plasma), urine and saliva are generally considered acceptable for estimating nicotine exposure.[13,14] Both urine and saliva could be matrices of choice for determining exposures to ETS in children since collection procedures are non-invasive, although it has been shown that urinary cotinine excretion varies considerably among persons exposed to similar amounts of nicotine.[15] Good correlations between nicotine intake and plasma cotinine levels in smokers and nonsmokers have been demonstrated[16] and, in addition, saliva cotinine concentrations have been shown to provide the same information as in plasma, although concentrations were found to be between 20 and 40% higher in saliva.[17] Etzel's review[15] of the relationship between cotinine levels and ETS exposure reported that saliva cotinine concentrations less than 10 ng/ml would usually result from ETS exposure without active smoking, although heavy passive exposure to tobacco smoke may produce levels in excess of this value. Pirkle *et al.*,[18] reporting the findings of the Third National Health and Nutrition Examination Survey (NHANES III) in the United States, found a median serum cotinine level of 0.526 ng/ml for adults who had reported some degree of ETS exposure either at home or at work. Hence assay sensitivity sufficient to quantitate levels lower than 0.5 ng/ml will be required in order to adequately assess very low level ETS exposures using cotinine as a biological marker. Bernert *et al.*[19] have recently reported the development and 'validation' of an assay for determining cotinine in human serum with a limit of detection of 0.05 ng/ml. From the data presented, this method was estimated to have a lower limit of quantitation (LOQ) in the region of 0.17 ng/ml.

The method described in this publication was validated for the simultaneous determination of cotinine and 3-hydroxycotinine in human saliva with lower limits of quantitation of 0.05 and 0.10 ng/ml respectively[20] and has been shown to be applicable to the quantitation of cotinine in subjects reporting low level ETS exposure.

Air monitoring studies

The determination of personal exposures to environmental tobacco smoke and respirable suspended particles (RSP) for housewives and office workers throughout Europe and South East Asia has been undertaken by these authors.[21–32] The studies involved subjects monitoring the air close to their breathing zone over 24 h periods using personal monitors. ETS particles were estimated using ultraviolet absorbing particulate matter (UVPM), fluorescing particulate matter (FPM) and solanesol related particulate matter (SolPM). Vapour phase ETS exposures were also assessed by simultaneous measurement of nicotine and 3-ethenylpyridine concentrations. Full details of the personal monitoring procedures and analytical methodologies applied in these studies have been published elsewhere.[21] Briefly, the subjects were categorised as either 'housewives' or 'office workers' and were

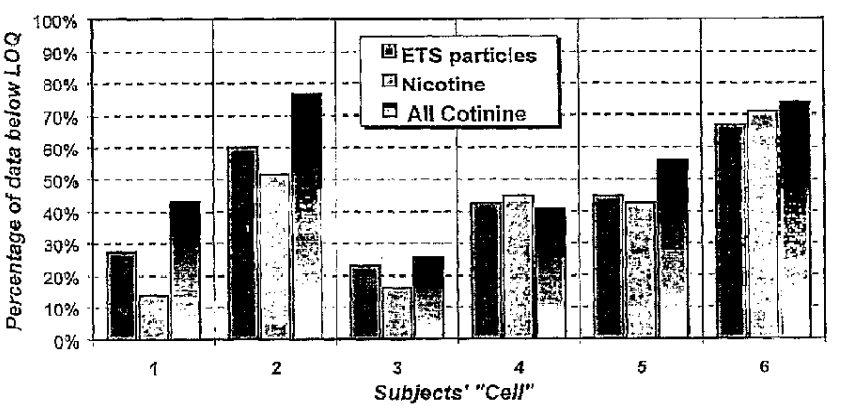

Figure 1 Percentage of analytical data below the limit of quantitation (LOQ). Cell 1: Housewives living in smoking households; Cell 2: Housewives living in nonsmoking households; Cell 3: Office workers living in smoking households and working in smoking workplaces; Cell 4: Office workers living in smoking households and working in nonsmoking workplaces; Cell 5: Office workers living in nonsmoking households and working in smoking workplaces; Cell 6: Office workers living in nonsmoking households and working in nonsmoking workplaces

further subdivided into 6 Cells (Figure 1) based upon the smoking status of their homes and/or workplaces. Housewives wore one personal monitor[33] and office workers two monitors (one at work and one at all other times) over a 24 h period from which a 24 h time weighted average (TWA) was calculated for all subjects. In addition, saliva cotinine concentrations were determined using a radioimmunoassay method with a LOQ of 1 ng/ml. The data for housewives studied in the European cities, based upon 24 h TWA concentrations, are listed in Table 1.

If data are normalised throughout Europe, the highest median 24 h TWA concentrations for ETS particles (SolPM) and nicotine were found for housewives living in smoking households. The highest median RSP concentrations for both smoking (71 $\mu g/m^3$) and nonsmoking homes (54 $\mu g/m^3$) were found in Turin. The highest median cotinine levels for housewives (2.9 ng/ml) were found in Stockholm. Table 2 compares similar data for Asian cities with those found for Sydney and overall median levels calculated for all the European cities investigated. For all the European cities combined, median 24 h TWA concentrations for ETS particles showed significant differences between nonsmoking homes (<0.26 $\mu g/m^3$) and smoking homes (4.1 $\mu g/m^3$). This trend was mirrored by similar differences for air nicotine concentrations (0.63 $\mu g/m^3$ *versus* <0.08 $\mu g/m^3$) and cotinine levels in saliva (1.4 ng/ml *versus* <1 ng/ml) determined for housewives living with smokers and nonsmokers respectively.

It should be noted from Tables 1 and 2 that the majority of measured cotinine concentrations are close to or below the LOQ (1 ng/ml) of the methodology used during the course of these investigations. Figure 1 depicts the percentage of results below the respective LOQs for ETS particles, nicotine and cotinine determinations. These are compared by Cell across Europe, which serves to demonstrate the inadequacy of using cotinine levels determined by this radioimmunoassay method as a biomarker for ETS exposure.

Personal monitoring or fixed site monitoring?

Methods chosen to assess exposures can ultimately depend upon the availability of suitable monitoring equipment. Fixed site monitors tend to be far easier to design and use, whereas personal monitors are of limited availability. Fixed site monitoring may also provide misleading information, especially in situations where the flow of air within an environment is not consistent and the positioning of the monitoring unit becomes critical. The integrity of results from personal monitoring may also be questionable if the design of the study as well as the equipment are not given serious consideration. In the case of monitoring infants and their exposures to any pollutants, including ETS, personal monitoring of the mother and fixed site monitoring of bedrooms and other locations within the home may be of major importance.

Table 1 Median 24 h TWA exposure concentrations for housewives in Europe

Location	RSP ($\mu g/m^3$)		ETS particles ($\mu g/m^3$)		Nicotine ($\mu g/m^3$)		Cotinine (ng/ml)	
	SH	NSH	SH	NSH	SH	NSH	SH	NSH
Stockholm	39	18	19	<0.26	1.1	<0.08	2.9	<1.0
Barcelona	63	51	11	0.98	0.74	0.11	1.4	<1.0
Turin	71	54	6.1	0.43	1.1	0.14	1.4	<1.0
Paris	62	36	3.3	0.62	0.52	0.13	1.3	<1.0
Bremen	36	25	0.13	<0.26	0.49	<0.08	1.4	<1.0
Lisbon	38	38	0.13	<0.26	0.19	<0.08	1.2	<1.0
Basel	34	28	1.4	<0.26	0.6	<0.08	1.0	<1.0
Prague	48	32	5.7	<0.26	0.72	0.15	1.2	<1.0

TWA: time weighted average; RSP: respirable suspended particles; ETS: environmental tobacco smoke; SH: smoking home; NSH: nonsmoking home

Table 2 Median 24 h TWA exposure concantrations for housewives in South East Asia, Australia and Europe

Location	RSP ($\mu g/m^3$)		ETS particles ($\mu g/m^3$)		Nicotine ($\mu g/m^3$)		Cotinine (ng/ml)	
	SH	NSH	SH	NSH	SH	NSH	SH	NSH
Hong Kong	45	46	<0.26	<0.26	<0.08	<0.08	<1.0	<1.0
Kuala Lumpur	52	48	<0.26	<0.26	0.18	<0.08	<1.0	<1.0
Beijing	102	70	8.4	0.55	1.4	0.15	1.0	<1.0
Sydney	30	24	0.92	<0.26	0.3	<0.08	<1.0	<1.0
Europe	52	34	4.1	<0.26	0.63	<0.08	1.4	<1.0

TWA: time weighted average; RSP: respirable suspended particles; ETS: environmental tobacco smoke; SH: smoking home; NSH: nonsmoking home

Table 3 Personal and fixed site monitoring of homes in Beijing, Hong Kong and Europe for respirable suspended particles ($\mu g/m^3$)

	Personal monitoring ($\mu g/m^3$)			*Fixed site monitoring* ($\mu g/m^3$)		
	Beijing	*Hong Kong*	*Europe*	*Beijing*	*Hong Kong*	*Europe*
Minimum	8.8	6.3	4.8	8.9	5.7	2.8
Maximum	364	93	555	592	134	4706
Median	69	41	36	92	55	25
Geometric mean	78	41	36	82	51	26

Table 4 Personal and fixed site monitoring of homes in Beijing, Hong Kong and Europe for environmental tobacco smoke particles ($\mu g/m^3$)

	Personal monitoring ($\mu g/m^3$)			*Fixed site monitoring* ($\mu g/m^3$)		
	Beijing	*Hong Kong*	*Europe*	*Beijing*	*Hong Kong*	*Europe*
Minimum	0.04	0.03	0.04	0.04	0.03	0.00
Maximum	2.5	0.57	10.4	2.9	0.59	23.4
Median	0.21	0.05	0.14	0.17	0.05	0.05
Geometric mean	0.28	0.07	0.2	0.2	0.07	0.13

During the studies conducted in Europe and Asia by these authors, variations in the concentrations of RSP and ETS particles have been noted in homes depending upon the collection method. Identical monitors were either worn by the housewives (personal) or located in fixed positions within each household. Tables 3 and 4 compare the results from the European cities overall with those for Hong Kong and Beijing. If median RSP levels are compared, personal monitors were found to give higher values than fixed site monitors in Europe, but lower values in Hong Kong and Beijing. For ETS particles, using the current methods, all results were close to or below the LOQ.

Low level cotinine determination

In order to improve the applicability of saliva cotinine measurements for estimating ETS exposure, a chromatographic method was developed and validated for the determination of both cotinine and 3-hydroxycotinine in this matrix with lower limits of quantitation of 0.05 and 0.10 ng/ml respectively. The method involved solid phase extraction of analytes from saliva, a procedure automated using Gilson ASPEC™ instrumentation (Anachem Ltd, Luton, UK), and subsequent liquid chromatographic separation prior to tandem mass spectrometric detection. Full details of the validation and analytical methodology have been reported elsewhere.[20] In order to provide evidence that the method was sufficiently sensitive to enable discrimination between varying degrees of ETS exposure, saliva samples were collected from nonsmoking subjects and subsequently analyzed. The subjects were of known nonsmoking status and had a variety of self-reported recent ETS exposure histories. Median concentrations of <0.05 ng/ml for self-reported unexposed nonsmokers, 0.65 ng/ml for nonsmokers reporting some ETS exposure and 1.28 ng/ml for nonsmokers living with smokers were found.

Another advantage of this assay was the ease with which a pre-screening schedule could be applied to collected saliva samples. In large scale ETS exposure studies a varying percentage of individuals will misreport their nonsmoking status, claiming to be nonsmokers when they are in fact smokers. A small portion of the saliva sample collected (10 μl) may be diluted with water prior to direct injection onto the mass-spectrometer. This simple screening procedure can easily distinguish between nonsmokers and smokers for any study requiring accurate verification of nonsmoking status.

The median value determined for subjects having reported as living with smokers was comparable with median values determined for housewives and office workers either living and/or working in smoking environments in the European and South East Asian personal monitoring studies performed by these authors (Table 2). The findings from this investigation demonstrate that saliva cotinine concentrations determined at sub-nanogram levels may have increased significance as a biomarker for ETS exposure. This may be of particular importance for the monitoring of children with parents or other family members who smoke. Methods with improved lower limits of quantitation are even more essential since lower cotinine levels resulting from reduced ETS exposures are more likely as parental awareness of the potential adverse health risks associated with ETS improves.

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References

1 United States Environmental Protection Agency. *Respiratory health effects of passive smoking: lung cancer and other disorders.* Washington DC, United States Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development. 1992; EPA/600/6-90/006F.

2 Etzel R, Pattishal E, Haley N, Fletcher R, Henderson F. Passive smoke and middle ear effusion among children in day care. *Journal of Paediatrics* 1992; **90**: 228–232.

3 Henderson R *et al.* Home air nicotine levels and urinary cotinine excretion in young children. *American Review of Respiratory Diseases* 1989; **140**: 197–201.

4 Tager IB *et al.* Effect of cigarette smoking on the pulmonary function of children and adolescents. *American Review of Respiratory Diseases* 1985; **131**: 752–759.

5 Weiss ST, Tager IB, Speizer FE, Rosner B. Persistent wheeze. Its relation to respiratory illness, cigarette smoking, and level of pulmonary function in a population sample of children. *American Review of Respiratory Diseases* 1980; **122**: 697–707.

6 Wu JM. Methods for measuring exposure to environmental tobacco smoke (ETS). *Journal of the Korean Society of Tobacco Science* 1997; **19**: 162–169.

7 Benowitz NL, Jacob P. Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. *Clinical Pharmacology and Therapeutics* 1993; **53**: 316–323.

8 Robinson DE, Balter NJ, Schwartz SL. A physiologically based pharmacokinetic model for nicotine and cotinine in man. *Journal of Pharmacokinetics and Biopharmacology* 1992; **20**: 591–609.

9 Benowitz NL. Use of biologic fluid samples in assessing tobacco smoke consumption. In: Grabowski J, Bell CS, (eds.) *Measurement in the analysis and treatment of smoking behavior (NIDA Research Monograph 48).* Government Printing Office, Washington DC, 1983.

10 Sheen SJ. Detection of nicotine in foods and plant materials. *Journal of Food Science* 1988; **53**: 1572–1573.

11 Davies RA, Stiles MF, DeBethizy JD, Reynolds JH. Dietary nicotine: a source of urinary cotinine. *Food Chemistry and Toxicology* 1991; **29**: 821–827.

12 Tunstall-Pedoe H, Woodward M, Brown CA. Tea drinking, passive smoking, smoking deception and serum cotinine in the Scottish heart health study. *Journal of Clinical Epidemiology* 1991; **44**: 1411–1414.

13 Jarvis MJ, Russell MAH, Benowitz NL, Feyerabend C. Elimination of cotinine from body fluids: implications for noninvasive measurement of tobacco smoke exposure. *American Journal of Public Health* 1988; **78**: 696–698.

14 Watts RR, Langone JJ, Knight GJ, Lewtas J. Cotinine analytical workshop report: consideration of analytical methods for determining cotinine in human body fluids as a measure of passive exposure to tobacco smoke. *Environmental Health Perspectives* 1990; **84**: 173–182.

15 Etzel RA. A review of the use of saliva cotinine as a marker of tobacco smoke exposure. *Preventative Medicine* 1990; **19**: 190–197.

16 Galleazzi RL, Daenens P, Gugger M. Steady-state concentration of cotinine as a measure of nicotine intake by smokers. *European Journal of Clinical Pharmacology* 1985; **28**: 301–304.

17 Curvall M *et al.* The pharmacokinetics of cotinine in plasma and saliva from non-smoking healthy volunteers. *European Journal of Clinical Pharmacology* 1990; **38**: 281–287.

18 Pirkle JL *et al.* Exposure of the US population to environmental tobacco smoke. The Third National Health and Nutrition Examination Survey, 1988 to 1991. *Journal of the American Medical Association* 1998; **275**: 1233–1240.

19 Bernert Jr. JT *et al.* Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Clinical Chemistry* 1997; **43**: 2281–2291.

20 Bentley MC *et al.* Validation of an assay for the determination of cotinine and 3-hydroxycotinine in human saliva using automated solid phase extraction and liquid chromatography with tandem mass-spectrometric detection. *Journal of Chromatography B* 1998. In press.

21 Phillips K, Bentley MC, Howard DA, Alván G. Assessment of air quality in Stockholm by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke. *Scandinavian Journal of Work, Environment & Health* 1996; **22** Supp 1: 1–24.

22 Phillips K *et al.* Assessment of air quality in Barcelona by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke. *Environment International* 1997; **23**: 173–196.

23 Phillips K, Howard DA, Bentley MC, Alván G. Assessment of air quality in Turin by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke. *Environment International* 1997; **23**: 851–871.

24 Phillips K, Bentley MC, Howard DA, Alván G. Assessment of air quality in Paris by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke. *Environment International* 1998; **24**: 405–425.

25 Phillips K, Howard DA, Bentley MC, Alván G. Measured exposures by personal monitoring for respirable suspended particles and environmental tobacco smoke of housewives and office workers resident in Bremen, Germany. *International Archives of Occupational and Environmental Health* 1998; **71**: 201–212.

26 Phillips K, Howard DA, Bentley MC, Alván G. Assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Lisbon by personal monitoring. *Environment International* 1998; **24**: 301–324.

27 Phillips K, Howard DA, Bentley MC, Alván G. Assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Basel by personal monitoring. *Atmospheric Environment* 1998; In press.
28 Phillips K, Bentley MC, Howard DA, Alván G. Assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Prague by personal monitoring. *International Archives of Occupational Health* 1998; 71: 379–390.
29 Phillips K, Howard DA, Bentley MC, Alván G. Assessment of environmental tobacco smoke and respirable suspended particle exposures of nonsmokers in Hong Kong using personal monitoring. *Environment International* 1998; 24: 851–870.
30 Phillips K, Bentley MC, Howard DA, Alván G. Assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Kuala Lumpur using personal monitoring. *Journal of Exposure Analysis and Environmental Epidemiology* 1998; In press.
31 Phillips K, Howard DA, Bentley MC, Alván G. Assessment by personal monitoring of respirable suspended particles and environmental tobacco smoke exposure for nonsmokers in Sydney, Australia. *Indoor and Built Environment* 1998; 7: 188–203.
32 Phillips K, Howard DA, Bentley MC, Alván G. Environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Beijing. *Indoor and Built Environment* 1998; In press.
33 Ogden MW *et al*. Personal monitoring system for measuring environmental tobacco smoke exposure. *Environmental Technology* 1996; 17: 239–250.